Reference: *Biol. Bull.*, **141**: 449–457. (December, 1971)

A STUDY OF HOMING BEHAVIOR IN THE LIMPET *SIPHONARIA ALTERNATA* [1]

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Individuals of several species of intertidal prosobranch and pulmonate limpets characteristically return to fixed locations or "homes" on rocks. These limpets move away from homes presumably to feed on algae present on rock surfaces and return to the same homes after feeding. Individual homes are usually marked by indentations or "scars" in the rock surface; the outer margins of scars often closely match the outline of the resident limpet's shell. The time of movement relative to the tidal cycle varies with the species of limpet.

Limpet homing has intrigued zoologists since the time of Aristotle (Arey and Crozier, 1921); how either prosobranch or pulmonate limpets find their homes has not been completely proven.

Four general hypotheses to explain limpet homing have been proposed. The first hypothesis is that limpets home by following clues external to their rocks (plane of polarization of light from the sky, sun or moon position, coastal landmarks, sky brightness). The second hypothesis is that animals use kinaesthetic information to navigate by a reverse-displacement or dead-reckoning system; this hypothesis is described more fully in a previous paper (S. Cook, 1969). The third hypothesis is that limpets home by using a topographic memory. The fourth hypothetical explanation is that animals follow clues which they themselves have created on rocks; such clues might include mucous trails or paths rasped in the algal cover of rocks.

Previous work has shown that the Hawaiian pulmonate limpet *Siphonaria normalis* continues to home without the use of either distant clues or reverse-displacement (S. Cook, 1969). In this paper I demonstrate that the pulmonate limpet *Siphonaria alternata* from the Florida Keys can home without using external clues, topographic memory, or reverse-displacement. I also present evidence that individual *S. alternata* home by following mucous trails.

General Materials and Methods

Field experiments

The *S. alternata* population used in all field experiments was located on small intertidal rocks on the seaward side of Ramrod Key, Florida.

Laboratory experiments

Individuals of *S. alternata* used in laboratory experiments were obtained from the Florida Keys on their original rocks. Most were collected from populations on

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Little Torch, West Summerland, and Big Pine Keys. All limpets were supplied by Mr. Stanley Becker (Tropical Atlantic Marine Specimens, P. O. Box 62, Big Pine Key, Florida).

Animals on their rocks were placed in a tidal aquarium system (Fig. 1). This system consists of two plastic aquaria connected by a discontinuous siphon. Water in the lower or reservoir tank is pumped by a Randolph peristaltic pump into a bell jar inverted above the level of the tidal aquarium. Water flows from the bell jar into the tidal aquarium through an incurrent siphon at a rate regulated by a burette attached to the siphon. When the tidal aquarium has filled to the high tide level, the discontinuous siphon begins to operate and water flows into the reservoir tank through a burette. After the tidal aquarium has emptied, an automatic timer triggers the Randolph pump to refill the bell jar and the cycle starts again. Two tidal cycles were used: (1) 6 hours of ebbing and low tide followed by 6 hours of rising and high tide per 12 hour period, and (2) 6 hours of ebbing and low tide followed by 18 hours of rising and high tide per 24 hour period. In both cycles rocks and limpets were uncovered for 2–3 hours at each low tide.

Illumination (12 hours per day) was provided by ambient fluorescent room lighting and a 100-watt incandescent bulb placed above the tidal aquarium. Aerated

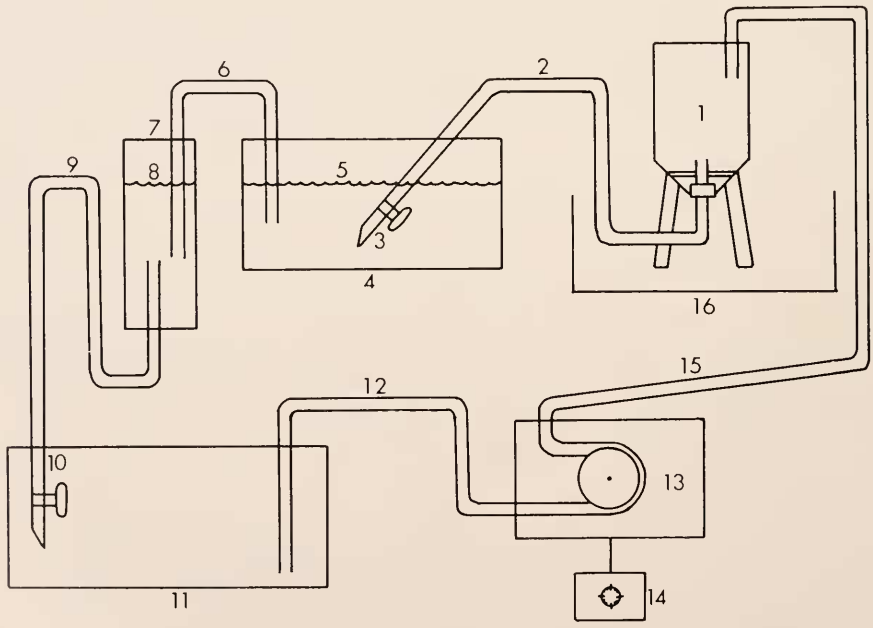

FIGURE 1. Diagram of tidal aquarium system: 1 = inverted bell jar, 2 = continuous incurrent siphon from bell jar to tidal aquarium, 3 = burette with stopcock, 4 = tidal aquarium tank, 5 = water level at which excurrent siphon is triggered, 6 = continuous siphon from tidal aquarium to plastic cylinder, 7 = plastic cylinder in which water level follows level in tidal aquarium, 8 = water level in plastic cylinder that triggers excurrent siphon, 9 = intermittant excurrent siphon from plastic cylinder to reservoir tank, 10 = burette with stopcock, 11 = reservoir tank, 12 = tubing from reservoir to Randolph pump, 13 = Randolph peristaltic pump, 14 = automatic timer, 15 = tubing from pump to bell jar, 16 = tank to catch overflow from bell jar.

"Instant Ocean" (Aquarium Systems, Inc., Wickliffe, Ohio) maintained at a salinity of 34–36‰ was used in the system; the water temperature varied from 22–25° C. The tidal aquarium was cleaned and refilled with fresh "Instant Ocean" every 6 to 14 days.

Only limpets which were active were chosen for laboratory experiments; no limpet which had been in the laboratory for more than 14 days was used.

Observations

Limpet behavior in the field and in the laboratory

In the field *S. alternata* moved away from homes when they were splashed by the incoming tide and when they became exposed to air on the ebbing tide. They returned to their homes by retracing their outbound paths when rocks were completely covered with water at high tide and when rocks began to dry out at low tide. Every limpet that I observed over a two week period homed. This behavior pattern is identical to that previously described for *Siphonaria japonica* (Ohgushi, 1955) and *Siphonaria normalis* (S. Cook, 1969).

Siphonaria alternata kept under artificial tidal conditions in the laboratory moved throughout the period of high tide and at low tide when rocks were damp; 71–76% of limpets moving during observation periods showed evidence of homing behavior. This percentage was considered sufficient to allow behavioral experiments in the laboratory.

Field experiments on the homing mechanism

The elimination of use of external clues in homing. Fifteen limpets were observed as they moved away from home scars; their approximate outbound paths were sketched on graph paper. When each animal began to reverse its heading at the end of its outbound trip, I rotated its rock 90° in the horizontal plane and recorded the limpet's path after rotation. I estimated the percentage of outbound path retraced by each individual.

All fifteen animals retraced 100% of outbound paths and entered their home scars. This indicates that *S. alternata* can home without using clues external to rocks.

The elimination of topographic memory as a homing mechanism. I followed outbound trips of 30 animals by drawing pencil lines along the sides of their outbound paths on rocks and sketching the approximate outlines of these paths on graph paper. After each animal had begun its return trip, it was removed from its own path and placed with its head adjacent to the freshly laid outbound path of another limpet on a topographically dissimilar rock. Each animal was replaced at a distance from the foreign scar equal to at least twice the length of its shell. In an attempt to offer each limpet only one foreign path to follow, I used only limpets in areas devoid of additional limpets. Paths of transplanted animals were followed and recorded; the percentage of foreign path followed by each displaced animal was estimated.

Twenty-two of the 30 animals retraced paths over unfamiliar topography and entered foreign scars. Four animals followed 70–80% of such paths but did not enter scars, while the remaining four did not move along the unfamiliar routes. This result indicates that use of topographic memory is not necessary for homing.

The elimination of reverse-displacement as a homing mechanism. A limpet moving along a reverse-displacement path should retrace its outbound path from its end to its beginning; the path of such an animal is shown in Figure 2b. Figure 2a represents the path of a limpet which does not follow a reverse-displacement path.

The records of 18 limpets from the preceding section were examined for evidence of reverse-displacement. In 10 of these cases, the headings of foreign paths were completely different from reverse-displacement headings. In the other 8 cases, initial headings necessary for limpets to turn onto foreign paths differed from initial headings necessary for reverse-displacement homing; after these initial differences both foreign paths and reverse-displacement paths were straight lines.

In the 10 cases in which paths were completely different, 7 animals followed foreign paths rather than reverse-displacement routes; the 3 remaining limpets did not follow the foreign trails, but rather took paths indistinguishable from reverse-displacement paths. In the 8 cases of initial difference, all animals turned to follow foreign paths. These results indicate that limpets can home without using reverse-displacement.

Laboratory experiments on homing

Ability to follow mucous trails laid on glass slides. Before use, 2″ × 3″ glass slides were cleaned with 10% (v/v) "7-X" solution, scrubbed with Alconox, and rinsed with distilled water. Grids were placed under Petri dishes filled with "Instant Ocean"; slides were placed in the Petri dishes and aligned with the grids. This allowed me to record the position of each animal throughout the experiment. Each animal was placed on a slide and allowed to lay a mucous trail; after it had done this, I removed it from the slide. The slide was rotated 90° within the dish and the water in the dish was changed. I then replaced each animal on the slide with its head next to its trail and with the long axis of its shell perpendicular to the trail. The distance from each limpet's head to either end of the trail was equal to at least twice the length of the limpet's shell. After each limpet's subsequent movements were recorded, its slide was placed in Alcian blue (0.1% (w/v) in 10% ethanol) for 1–5 minutes to stain the mucous trails. The length of each animal's

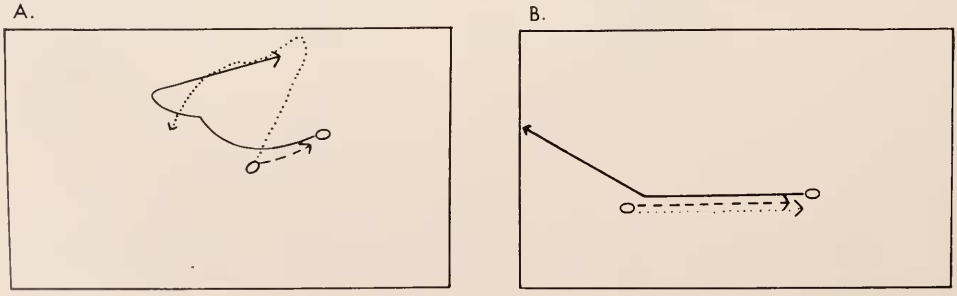

Figure 2. Actual paths of two limpets compared with paths predicted by reverse-displacement; Solid line: original path of the animal; Dashed line: path of the animal after replacement; Dotted line: path after replacement predicted by reverse-displacement; (A) represents a limpet which did not move along a path predicted by reserve-displacement; (B) shows a limpet that moved along a path predicted by reverse-displacement.

TABLE I

Lengths of trails available for following in laboratory experiments

Experiment	Lengths of trails available in cm		Length of trail/ length of limpet shell	
	Range	Mean	Range	Mean
1	1.3–3.8	1.8	1.4–6.0	2.6
2	1.3–6.4	1.9	1.0–8.0	2.9
3 Own trails	1.0–3.8	2.0	1.6–6.0	3.2
Foreign trails	1.0–3.8	1.5	1.3–6.0	2.5

original trail and the distance that the animal's second trail overlapped the original were determined from grid records. I then computed the percentage of the original trail that each limpet retraced and compared paths followed after replacement with paths predicted by reverse-displacement.

Forty of forty-six limpets retraced 90–100% of their original trails (Fig. 3a). Lengths of trails available for following are in Table I. Thirty-three of the forty-six animals (72%) did not follow reverse-displacement paths. Before slides were stained with Alcian blue, macroscopic spots of mucous were visible at the beginnings of trails. These spots stained dark-blue; staining rendered visible, with a dissecting microscope, granules and streaks scattered along the length of each trail.

These results indicate that limpets can retrace mucous trails on glass slides which lack obvious topography without the use of clues external to the slides and reverse-displacement. Animals also can retrace their movements in the absence of paths rasped in algal cover.

Elimination of random movement and use of grid lines in trail following. It is possible that limpets in the preceding experiment may not have followed mucous trails. Rather they may have used the pattern of grid lines in some way; alternatively, when limpets lay short trails, apparent trail following may result from random movements. A simple test of these possibilities would be to remove a limpet from the end of a mucous trail, turn the slide over, and replace the animal on the clean surface at a point on the grid adjacent to its original trail as in the preceding experiment. If the animal does not retrace the path of the mucous trail, the above alternatives can be discounted.

Forty-seven limpets were tested in this way. After I removed each limpet from its trail, I selected an arbitrary point for replacement on the grid next to its trail. Slides were rotated 90° and then turned over. I changed the water in each dish, replaced each animal on the clean glass with its head at the pre-selected point, and recorded its subsequent path. I then measured the length of the original trail and the distance that the path after replacement overlapped that of the trail. From this, I computed the percentage of the path of the trail that was apparently retraced by each animal after slide inversion.

A much smaller proportion of animals (9 of 47) retraced 90–100% of their original paths (Fig. 3b) than did animals in the previous experiment (Fig. 3a). Lengths of original paths are given in Table I. The means of the distributions of percentages of trails followed in the two experiments were significantly different

($P < 0.001$, two-tailed t-test). Trail following therefore can not be explained in terms of random movement or use of grid patterns as clues; limpets apparently do detect and follow mucous trails.

Elimination of use of minor topographic features. It is possible that limpets may retrace subtle topographic features present on individual slides instead of following mucous trails. To examine this, 15 pairs of snails were allowed to lay trails on separate glass slides. Each member of a pair was then removed from its slide and replaced next to the trail of its partner. Movements before and after replacement were plotted. After each test run was finished, each animal laid another trail on a clean area of its original slide and was tested for the ability to follow it. The percentage of each kind of trail followed was calculated for each animal.

Animals followed trails of other individuals about as often as they followed their own trails (Fig. 4). Information on trail lengths is in Table I. The means of the distributions of the percentage of trails followed for the two situations were not significantly different ($P = 0.90$, two-tailed t-test). Results obtained when animals were placed on foreign trails were similar to the results of the field experiment in which limpets were placed on foreign paths ($P > 0.90$).

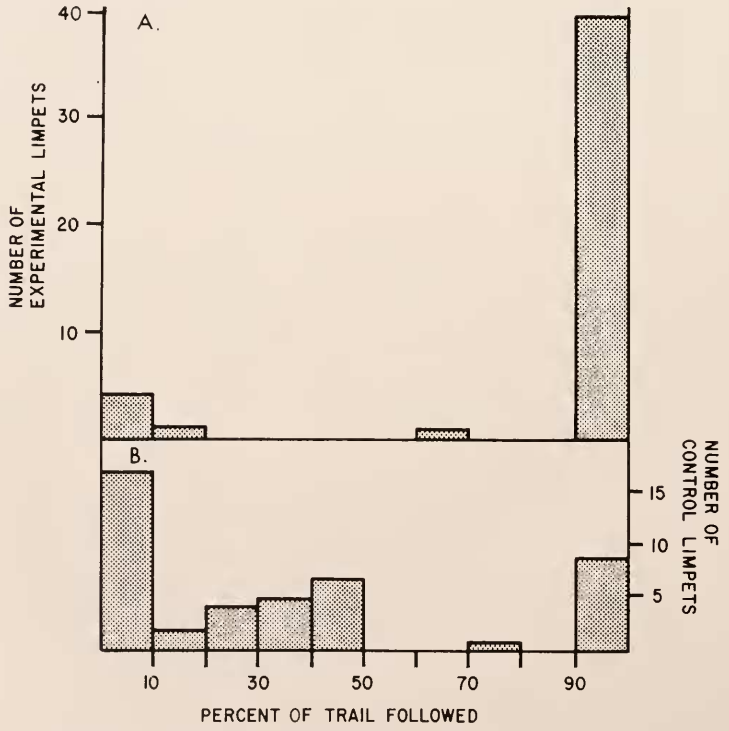

FIGURE 3. Ability of *S. alternata* to follow mucous trails; (A) experimental animals were placed next to paths of actual mucous trails; (B) control animals were placed on inverted slides in the same relative positions to trails as experimentals, except that no trails were present (see text).

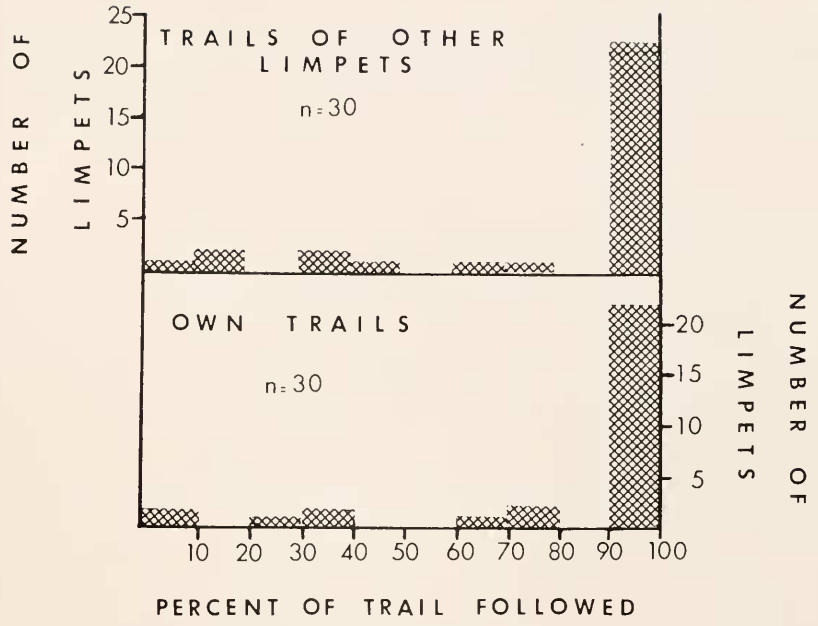

FIGURE 4. Ability of *S. alternata* to follow trails laid on slides by other individuals compared to their ability to follow their own trails.

These results indicate that animals can follow mucous trails laid by other limpets on glass slides. Memory of minor topographic irregularities that may exist on slides is therefore unnecessary for trail-following.

DISCUSSION

Results of field experiments show that *S. alternata* can home without using either distant clues, reverse-displacement or topographic memory. Laboratory experiments indicate that limpets are capable of following mucous trails (1) in the absence of major or minor features of topography, (2) in the absence of radula markings in algal cover, and (3) under conditions which should eliminate use of reverse-displacement and distant clues. The following of mucous trails laid by the limpets themselves on rocks is the simplest explanation for homing consistent with these results.

Inertial navigation is a possible means of homing that has not been considered in previous studies of limpet behavior. To home by such a mechanism an organism must have some means of measuring linear and angular acceleration as well as possess an internal clock (Barlow, 1964). The paired statocysts of pulmonate mollusks can probably detect accelerations of the magnitude of gravitational accelerations (*ca.* 980 cm/sec^2) (Charles, 1966); there is no evidence that these organs can detect the much smaller accelerations likely to be encountered in limpet homing. The fact that limpets can home to unfamiliar scars using foreign trails may indicate that such a mechanism is unnecessary for homing.

Mucous trail following is probably used by other species in the genus *Siphonaria* in homing. *Siphonaria normalis* in Hawaii can home without using external clues or reverse-displacement and must either detect information created on rocks during feeding excursions or use topographic memory (S. Cook, 1969). *Siphonaria atra, S. sipho,* and *S. japonica* all appear to home by moving along sides of previous outbound paths (Abe, 1940); this suggests that they are also following trails.

The mechanism of homing in the prosobranch limpet genus *Patella* may be similar to that used by *S. alternata*. Funke (1968) has found that several species of *Patella* follow clues that they have made on algal-covered glass plates in order to return to homes established on the plates. He concluded that limpets follow chemical clues in homing; he did not, however, consider the alternate possibilities that animals may follow radula scrapings in algal cover or may follow mucous trails by moving along textural discontinuities provided by the mucous. In a recent field study of homing in *Patella vulgata, P. depressa* and *P. aspersa*, A. Cook, Bamford, Freeman and Tiedeman (1969) have shown by rock rotation and displacement experiments that use of external clues and reverse-displacement are not necessary for homing; these results agree with Funke's (1968) explanation of homing. These authors also found that some limpets continued to home after rock surfaces around homes were scrubbed with wire brushes or treated with NaOH; they further report that chiselling trenches in rocks between limpets and homes did not prevent return. These results do not support Funke's conclusions; however, Cook *et al.* state that their experiments may not have eliminated all topographic clues or clues created by limpets.

Limpets may follow mucous trails either by following physical discontinuities of some sort created by mucous streaks or by following chemical clues. Possible chemical clues fall into 2 general categories: (1) attached, non-diffusible chemical clues such as those proposed for barnacle substrate selection during larval settling (Crisp and Meadows, 1963) and (2) chemical clues that diffuse out of trails.

In the laboratory *S. alternata* can follow trails after the trails have soaked for 48–49 hours in sea water, but do not follow trails soaked for 68–76 hours (S. Cook, 1970); experiments on the duration of effective trails on rocks in the field are lacking. It would seem likely that trails persisting for similar periods in the field would become covered with bacteria and other detritus; such accumulated debris would presumably alter the texture of any physical discontinuities characteristic of trails and might at least partially mask attached chemicals. If persistent trails exist which can be followed, this would suggest that limpets do not depend on physical discontinuities for homing clues; the probability that attached chemicals could be detected would also be decreased. Persistence of effective trails would not eliminate the use of diffusible chemicals. If such chemicals are involved, they must either (1) be present in large enough quantities so that they are detectable for 48 hours, (2) be packaged in the mucus and slowly released into the water over a period of time, or (3) not be released until limpets retrace trails. In the latter case limpets might release chemicals by the action of salivary enzymes that break down mucus; alternatively the physical action of limpet grazing might release such chemicals.

I am most grateful to S. A. Wainwright and my husband, Clay Cook, for their helpful suggestions and editorial advice throughout this work. I was supported during this study by a National Science Foundation Graduate Fellowship; my field work in the Florida Keys was also supported by a Summer Scholarship from the Duke University Graduate School.

Summary

1. The pulmonate limpet *Siphonaria alternata* returns consistently to fixed "home" positions on intertidal rocks when rocks are completely covered with water at high tide and when rocks begin to dry out at low tide.

2. Limpets homed after rock rotation, were able to follow paths made by other limpets on foreign rocks, and did not follow paths characteristic of reverse-displacement. These results eliminate use of external clues, topographic memory, and reverse-displacement and indicate that limpets home by using clues that they have previously created on rocks.

3. Limpets can follow mucous trails that they have previously laid on clean glass slides in the laboratory. This result indicates that limpets can follow mucous trails without using paths made by radula marks in algal cover. This behavior does not result from random movements.

4. The above results support the hypothesis that homing *S. alternata* retrace mucous trails that they have laid previously.

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